Communication

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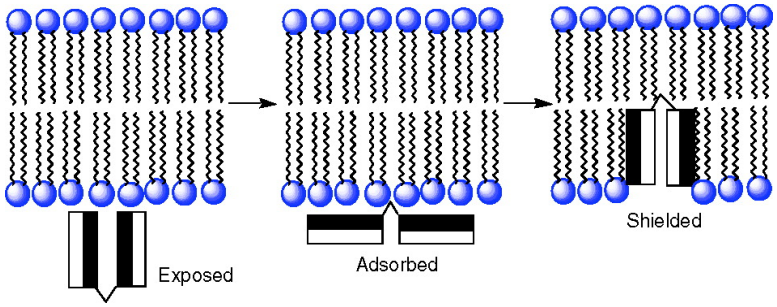

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Persulfated Molecular Umbrellas as Anti-HIV and Anti-HSV Agents

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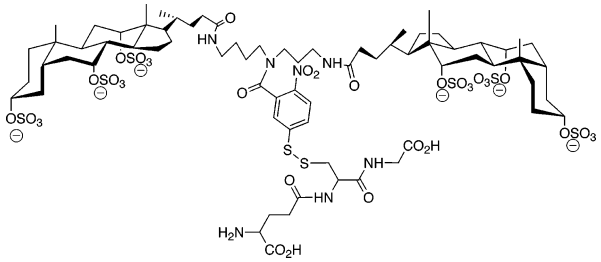
Previous studies from our laboratory have shown that a di-walled molecular umbrella, bearing three sulfate groups on each of two choloyl moieties, is capable of crossing phospholipid bilayers (Chart 1).[1] On the basis of its facial amphiphilicity, we postulated that

Chart 1



membrane transport was likely to occur via an umbrella mechanism, that is, where the conjugate crosses the membrane in a shielded conformation (Figure 1).[2] Recently, we hypothesized that molecular

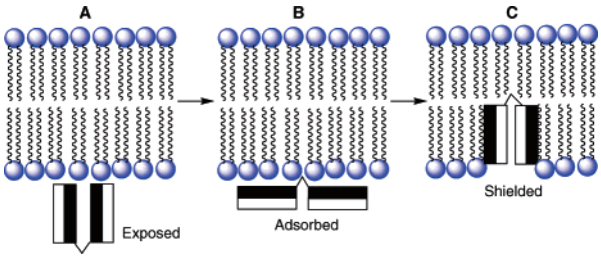
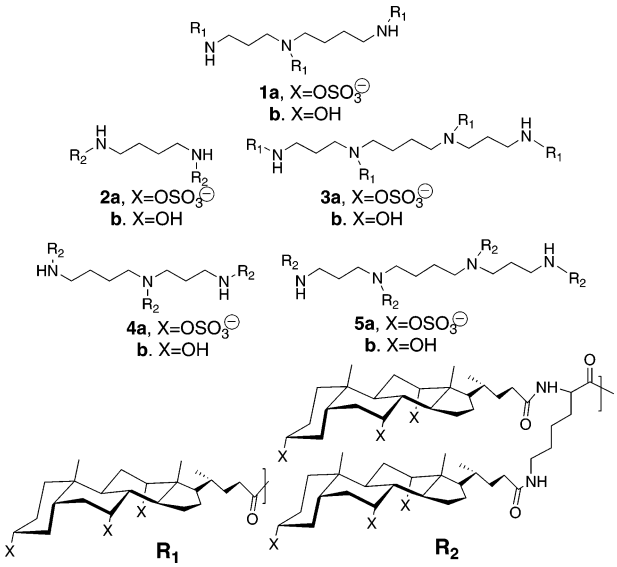


Figure 1. Stylized illustration of a di-walled molecular umbrella being drawn into a lipid bilayer from an initial exposed (A) to an adsorbed (B) to a shielded (C) state. Here, the shaded and unshaded rectangles represent hydrophobic and hydrophilic faces of the molecular umbrella, respectively. For simplicity, an attached polar agent (e.g., glutathione) has been omitted.

umbrellas of this type might have potential as anti-HIV and anti-HSV agents. Specifically, we reasoned that since anionic polymers such as dextran/dextrin sulfate and cellulose sulfate are known to inhibit cellular binding of HIV and HSV by competing for viral envelope glycoproteins (both HIV and HSV are lipid-enveloped viruses), that persulfated molecular umbrellas might behave similarly.[3] Moreover, the potential for crossing hydrophobic barriers (e.g., the blood-brain barrier) raises the possibility that such compounds could function as novel agents for therapeutic as well as prophylactic use.[4]

Motivated by this rationale, we have carried out a structure−activity investigation with a series of persulfated molecular umbrellas containing different numbers of amphiphilic units, that is, **1a**, **2a**, **3a**, **4a**, and **5a** (Chart 2). In this communication, we show that significant anti-viral activity by these conjugates has been found,

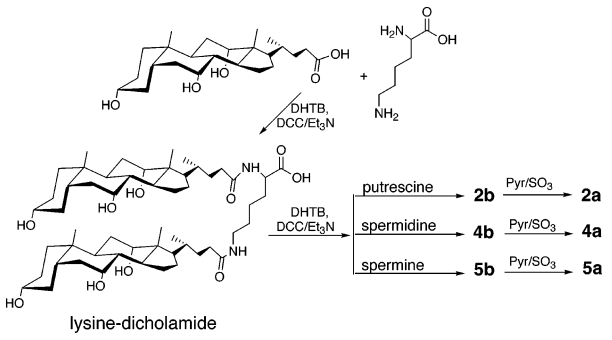
Chart 2



especially for those having higher numbers of facially amphiphilic units, that is, with **4a** and **5a**. We further show that despite its size, **5a** is capable of crossing phospholipid bilayers. The unique combination of anti-viral activity, an ability to cross hydrophobic barriers, a lack of cytotoxicity, and a simple three-step synthesis from biogenic starting material suggests that **5a** and related conjugates may be exploitable as a novel class of anti-viral agents for systemic and topical applications.

Hydroxylated precursors **1b** and **3b** were prepared by condensing cholic acid with spermidine and spermine, respectively.[5,6] Acylation of lysine with cholic acid, to give lysine-dicholamide, followed by condensation with putrescine, spermidine, and spermine, afforded **2b**, **4b**, and **5b**, respectively (Scheme 1). Using sulfation methods

Scheme 1



similar to those used for the preparation of **3a**, we then synthesized conjugates **1a**, **2a**, **4a**, and **5a**.[6] As expected, each conjugate exhibited excellent water solubility.

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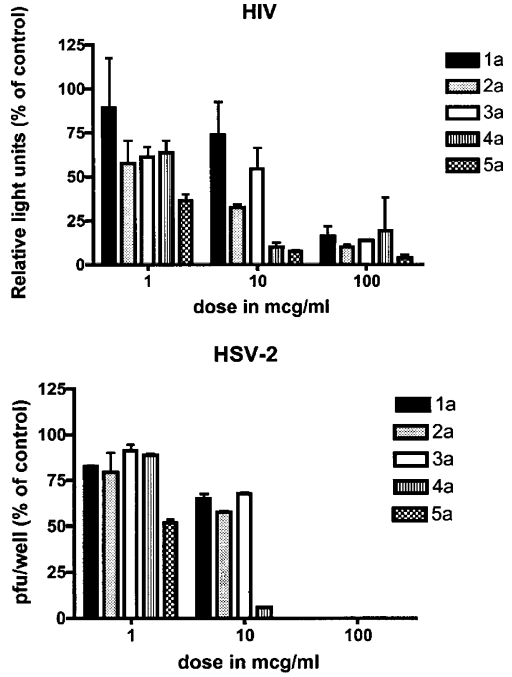

Figure 2. Bar graphs showing anti-HIV-1 and anti-HSV-2 activities.

The anti-HIV activity of these conjugates was then measured using U87.CD4.CCR5 cells, replication-defective HIV-1 virus and a luciferase assay as previously described.[7,8] At a dosage of 1 μg/mL, **5a** showed moderate activity; **2a**, **3a**, and **4a** were less active; and **1a** was inactive (Figure 2). At 100 μg/mL, all conjugates showed significant activity, and inhibition was >95% with **5a**. The anti-HSV activity was also compared by conducting plaque reduction assays with HSV-2(G) and human cervical epithelial cells (CaSki).[8] At a concentration of 100 μg/mL, all of the conjugates completely inhibited HSV-2 (not shown). At a concentration of 10 μg/mL, complete and very significant inhibition was found for **5a** and **4a**, respectively. In contrast, the activities of **1a**, **2a**, and **3a** were only modest at this concentration.

To confirm that **5a** can cross hydrophobic barriers, we prepared vesicles (200 nm, extrusion) from 1-palmitoyl-2-oleyol-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol (POPG) [POPC/POPG, 95/5, mol/mol], using an aqueous solution that was 0.500 mM in **5a** and 1.66 mM in glutathione (GSH). In this experiment, GSH was used as a marker to quantify the percentage of the aqueous phase that was captured by the vesicles since it neither crosses, nor absorbs to, the membranes of POPC/POPG vesicles.[2,9] After 52 h of dialysis at 23 °C, analysis of the dispersion showed that 6.2% of GSH was captured. In contrast, this same dispersion was found to contain 16.4% of **5a**, indicating that there is significant absorption as well as entrapment of the molecular umbrella. Whereas extended dialysis did not change the percent of entrapped GSH, a first-order decrease in **5a** was observed (Figure 3). The fact that the percentage of **5a** drops to a level that is well below that of GSH clearly indicates that the former is escaping from the vesicle interior, that is, it crosses the bilayer.

In preliminary studies, we have found that **5a** inhibits both binding of HSV to cell surfaces and penetration. Although the former was predicted on the basis of the ability of sulfated or sulfonated polymers to competitively block binding of HSV to cell surface heparan sulfate, the substantial activity of post-binding was unexpected. Our working hypothesis is that **5a** associates with fusogenic segments of glycoproteins of HSV via hydrophobic and ionic interactions, which reduces their conformational freedom and

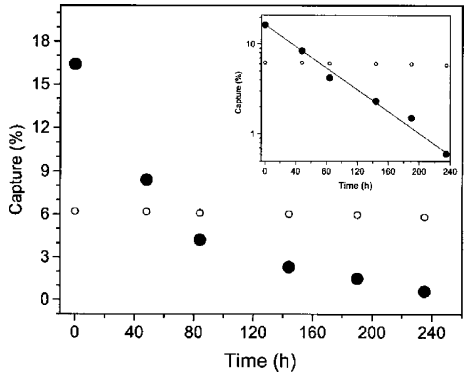

Figure 3. Plot of percent of GSH (○) and **5a** (●) that is captured by POPC/POPG vesicles as a function of dialysis time at 23 °C, after an initial dialysis period of 52 h. Inset shows a semilogarithmic plot of these data. Error values lie within the data points themselves.

their ability to "reshuffle" the surrounding lipids that is necessary for membrane fusion. It is also noteworthy that **5a** has been found to be noncytotoxic toward CaSki cells. Human cervical cells were exposed to serial dilutions of **5a** (in the absence of virus) overnight (acute) or for 3 h each day for 4 consecutive days (chronic) to examine the effects on cell growth and viability using an MTS assay. No cytotoxicity was observed following chronic or acute exposure even at concentrations as high as 1000 μg/mL.

In a broader context, the results reported herein illustrate a new approach to designing drugs that are able to cross membranes: For passive transport across lipid bilayers, rigid molecules must be reasonably small, and their hydrophilic/lipophilic properties must be within narrow boundaries.[10] The molecular amphomorphism of umbrella compounds largely circumvents these restrictions, allowing the very large **5a** (6265 Da) to escape from vesicles under conditions in which the small GSH (307 Da) cannot.

Further studies that are currently in progress are aimed at (i) clarifying the influence that the number of sterols versus the number of sulfate groups has on anti-viral activity, (ii) gaining greater insight into the mechanism by which **5a** operates, and (iii) exploiting this chemistry for biomedical applications.

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Supporting Information Available: Procedures for synthesizing **1a**, **2a**, **4a**, and **5a** and capturing of GSH and **5a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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